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LEAF OPTICAL RESPONSES TO LIGHT AND SOIL NUTRIENT AVAILABILITY IN TEMPERATE DECIDUOUS TREES[1]

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Leaf optical parameters influence light availability at the cellular, leaf, and canopy scale of integration. While recent studies have focused on leaf optical responses to acute plant stress, the effects of changes in plant resources on leaf optics remain poorly characterized. We examined leaf optical and anatomical responses of five temperate deciduous tree species to moderate changes in nutrient and light availability. Spectral reflectance in the visible waveband generally increased at high light, but decreased with increased nutrient availability. Patterns of both spectral reflectance and absorptance were primarily determined by chlorophyll concentration although carotenoid concentration was also influential. While most anatomical features did not explain residual variation in reflectance, cuticle thickness was significantly related to reflectance at complementary angles compared to the angle of incidence. Absorptance did not change with light environment; however, absorption efficiency per unit biomass increased by approximately 40% under low light, due to reduced leaf mass per area. We conclude that changes in resource availability differentially influence leaf optical properties and that such changes are driven primarily by changes in pigment concentrations. The magnitude of leaf optical responses to moderate changes in resource availability was comparable to those of acute stress responses and varied among species.

Key words: absorption efficiency; acclimation response; carotenoids; chlorophyll; cuticle; leaf mass per area; reflectance; resource availability.

The optical properties of leaves influence the availability of photosynthetically active radiation at the cellular level, the penetration of light through plant canopies, and ultimately the patterns of radiative balance and light attenuation in vegetated ecosystems. In addition, spectral reflectance patterns of leaves are of central interest in remote sensing applications, particularly remote assays of the physiological status of plants. A great deal of research has focused on responses of leaf optical characteristics to plant ''stress'' factors, such as exposure to gaseous pollutants (Carter et al., 1992, 1995), increased temperature and CO_2 (Carter et al., 2000), heavy metal exposure (Schwaller et al., 1983), UV-B radiation (Bornman and Vogelmann, 1991), and other abiotic stress agents such as drought and extreme nutrient deficiency (Carter et al., 1989; Carter, 1993, 1994; Zhao et al., 2003). In contrast, few studies have addressed leaf optical responses to differences in plant resource levels that do not involve acute stress responses, and consequently, analyses of plant responses to moderate changes in light and nutrient levels in variable natural environments are few. To date, studies on acclimation responses of leaf optics have mainly addressed leaf reflectance patterns as a proxy measure of leaf chlorophyll (Blackburn, 1999), N (Filella and Penuelas, 1994), xanthophyll cycle and other carotenoid pigments (Gamon et al., 1997; Gitelson et al., 2002), and non-chromophore-containing biochemical constituents (Fourty and Baret, 1998).

Leaf spectral reflectance and transmittance patterns in the visible range are thought to be determined mainly by chlorophyll concentrations (Vogelmann, 1993). Chlorophyll concentrations commonly decrease in response to increased ambient light conditions (Björkman, 1981; Givnish, 1988), reflecting in part changes in N allocation to favor dark cycle enzymes such as ribulose bisphosphate carboxylase/oxygenase (Rubisco). Such a response is predicted to be associated with increased leaf reflectance, though few data exist to test this prediction. Poorter et al. (2000) found that five climax species in Venezuela had similar spectral reflectance but lower transmittance values in sun vs. shade leaves resulting in slightly higher absorptance in the sun leaves; however, absorption efficiency (absorptance per unit leaf mass) was substantially greater in the shade leaves. Conversely, in a study examining two Southeast Asian *Hopea* species, absorptance and chlorophyll content increased in plants grown in low light as compared to those in medium or high light (Lee et al., 2000).

Acclimation to high light also often results in increases in cuticular thickness, whose primary function is prevention of water loss (reviewed in Grace and van Gardingen, 1996). Such cuticular changes may also alter leaf surface reflectance patterns (Grant et al., 1993; Barnes and Cardosa-Vilhena, 1996). Therefore, if reflectance does in fact increase with light, this response may be partially attributed to cuticular changes.

Increased availability of soil nutrients, in particular N, is commonly associated with increased leaf N and chlorophyll content (Johnson et al., 1997). Such a response should thus result in decreased leaf reflectance. The combined effects of altered light and nutrient resources on leaf optical properties are a matter of speculation. One might conjecture that responses to light conditions might be greater than those to nutrient availability, given that light may affect leaf reflectance via several mechanisms (e.g., N allocation shifts, photoprotection, photobleaching, and changes in leaf surface characteristics).

In the present study, we compared leaf optical characteristics in five temperate deciduous tree species grown in a 2 × 2 factorial experiment consisting of high and low light and nutrient conditions. The main questions we addressed are as

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follows: (1) How do leaf optical properties respond to moderate changes in light and nutrient resources? (2) To what extent are changes in leaf chlorophyll and carotenoid concentrations responsible for observed differences in leaf optical properties? (3) Are the effects of light and nutrients on leaf optics additive? (4) What contribution does leaf anatomy make to responses of leaf optical properties?

MATERIALS AND METHODS

Experimental design and plant growth conditions—We selected five temperate deciduous tree species for our study, *Prunus serotina* Ehrh. (black cherry), *Acer saccharum* Marsh. (sugar maple), *Acer rubrum* L. (red maple), *Quercus rubra* L. (red oak), and *Fraxinus americana* L. (white ash), all of which are common to forests in the Great Lakes–St. Lawrence region of eastern Canada. Saplings were of local provenance obtained from 2-yr-old bareroot stock. In February 2001, 20 saplings of each species were potted in 21.5 cm diameter pots in soil (silty sand loam) obtained from the Koffler Scientific Reserve at Joker's Hill (44°03′ N, 79°29′ W) in the Oak Ridges Moraine just west of Newmarket, Ontario, where a corresponding field study was being conducted on a similar suite of species.

Experiments were conducted under controlled glasshouse conditions at the Faculty of Forestry, University of Toronto, Toronto, Ontario, Canada. As the experiment was initiated in February, daylight was supplemented with sodium halide lamps from 06h00 to 20h00 for the initial months. Daytime temperatures were maintained between 25–30°C. Saplings were watered daily at 07h00 by an automatic watering system, supplemented with hand-watering on particularly hot days to avoid water stress.

The experiment examined the influence of moderate changes in light and nutrient availability on leaf optical and anatomical properties through the use of a 2 × 2 factorial design consisting of high and low light and nutrient conditions. Plants in the high light treatment received a maximum photosynthetic photon flux density of approximately 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while plants in the low light treatment received approximately 240 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 30% of the light available in the high light treatment. The shade treatment was provided by neutral density shade cloth covering a wooden frame. Each sun or shade block contained only one replicate per species per nutrient treatment. The high nutrient treatment was achieved using 6-month slow-release fertilizer pellets (16-10-10, Nutricote, Plant Products Co. Ltd., Brampton, Ontario, Canada) that enhanced nutrient availability by a rate of 150 kg $N \cdot ha^{-1} \cdot y^{-1}$, a moderate increase within the range of nitrogen mineralization rates in Northern temperate forests (Groffman et al., 1993, 2001). Saplings were randomly allocated to treatment and initial bench position. Each month, sapling positions were rotated to avoid confounding effects of temperature or light gradients within the glasshouse. For the winter period of October 2001–March 2002 all saplings were placed outside in the courtyard and their pots insulated with woodchips. Measurements were made in July 2002 after saplings had been in treatment for one and a half years.

Leaf optical measurements—Leaf optical measurements were made using a custom-built dual integrating sphere system. We used an ISP-REF integrating sphere (Ocean Optics, Inc., Dunedin, Florida, USA) with a built-in collimated light source and gloss-trap in combination with a FOIS-1 fiber optic integrating sphere (Ocean Optics). Both integrating spheres were connected via fiber optic bundles to a S2000 UV-VIS-shortwave NIR spectroradiometer (Ocean Optics) with a resolution of approximately 0.5 nm. The two integrating spheres were aligned using a rack-and-pinion slide (NT61–285, Edmund Industrial Optics, Barrington, New Jersey, USA) with a platform for each sphere. By clamping a leaf between the two integrating spheres, we were able simultaneously to measure leaf spectral reflectance and transmittance. Three to five individuals per species and treatment were measured. Sample size varied as a result of differential winter mortality across species and treatments. Three leaves per plant were measured at different locations in the sapling canopy. Total spectral reflectance in the visible range (400–700 nm) was calculated as $(R_s - R_d)/(R_r - R_d)$, where R_s is the light reflected from the leaf surface, R_d is the dark reference measuring stray light within the ISP-REF sphere when no leaf is present and no illumination supplied, and R_r is the ISP-REF sphere output when a white reference standard (Edmund Industrial Optics) is illuminated. Spectral transmittance was calculated similarly as $(T_s - T_d)/(T_r - T_d)$, where T_s is transmittance of light through the leaf sample, T_d is stray light within the FOIS-1 sphere when no leaf is present and no illumination supplied, and T_r is the FOIS-1 sphere output when no leaf is clamped between the two spheres. Absorptance was calculated as $A = 1 - (R + T)$. To ensure that measurements were not biased due to radiation transmission between the ISP-REF and FOIS-1 spheres, we made dual integrating sphere measurements as described above as well as single integrating sphere measurements using each sphere separately on a range of colors of paper with homogenous coloration. No difference existed between dual versus single integrating sphere measurements in the visible range ($y = x$, $R^2 > 0.99$).

A second set of measurements were made using the gloss-trap in the ISP-REF integrating sphere. The gloss-trap functions to remove light reflected from the leaf surface at an angle complementary to that of the incident radiation by opening a port through which this radiation can escape. Specifically, the ISP-REF light source is positioned at 4° and the gloss-trap at −4° from the opening exposing the leaf surface. Light reflected at complementary angles to the angle of incidence is considered to consist primarily of specularly reflected light (light reflected at the air–cuticle interface). However, there was a strong chlorophyll signal in the supposedly specular component ($R_{including} - R_{excluding}$, where $R_{including}$ is all light reflected by the leaf and $R_{excluding}$ is light reflected at all angles not complementary to the angle of incidence), indicating that our measurements were capturing a substantial amount of diffuse reflectance given that specularly reflected light should be spectrally flat (Grant, 1987; Grant et al., 1993). Measurement of a white reflectance standard (Edmund Industrial Optics) with the gloss-trap open and shut indicated that the gloss-trap was capturing between 10–15% diffuse radiation, substantially overestimating the specular component and accounting for the strong chlorophyll signal in our measurements. This suggests that the gloss-trap method is not adequate for the quantitative measurement of specular reflectance from leaves. It may, however, be the case that this method could be an easily measured correlate of specular reflectance and may also be used in parameterization of bidirectional reflectance canopy models (Breon et al., 2002).

After the optical measurements, chlorophylls and carotenoids were extracted from two leaf discs per leaf with N,N-dimethylformamide (DMF) and measured spectrophotometrically (Wellburn, 1994). Fresh leaf area was then measured, and the leaves then dried at 60°C for 2 days and weighed for calculation of leaf mass per area (LMA).

Leaf anatomy measurements—Free hand sections were made on each sample leaf. Each section was wet mounted and immediately examined using a Reichert-Jung Polyvar microscope (Leica Microsystems, Nussloch, Germany). Digital photographs were taken of each section at 10× and 40× with a Nikon Coolpix 900 digital camera (Nikon Corporation, Tokyo, Japan) attached to the microscope (Fig. 1). The thicknesses of the cuticle, adaxial and abaxial epidermises, palisade and spongy mesophyll layers, and total leaf were measured using the public domain Image J program (US National Institute of Health, Bethesda, Maryland, USA). The photographic scale was calibrated using a standard ocular micrometer slide.

Statistical analysis—The experimental treatments were implemented as a 2 × 2 factorial complete randomized block design. Analysis of variance (ANOVA) (PROC GLM, SAS version 8.1, SAS Institute, Cary, North Carolina, USA) was used to test for species and treatment differences. Independent variables included light, fertilization, and species. Dependent variables included absorptance (%A), total reflectance ($\%R_{total}$) and transmittance (%T) between 400–700 nm; chlorophylls *a* and *b*; total chlorophyll and carotenoids; cuticle, adaxial and abaxial epidermal, palisade and spongy mesophyll, and total leaf thicknesses; and absorption efficiency. Absorption efficiency was calculated as %A/mg chlorophyll and %A/g biomass. Both efficiency measures were used as dependent variables. To meet assumptions surrounding ANOVA, all leaf anatomical characteristics, tissue density, $\%R_{total}$ and %T were log transformed and %A square root-arcsine transformed. To examine the relationship between chlorophyll concentration and absorptance or reflec-

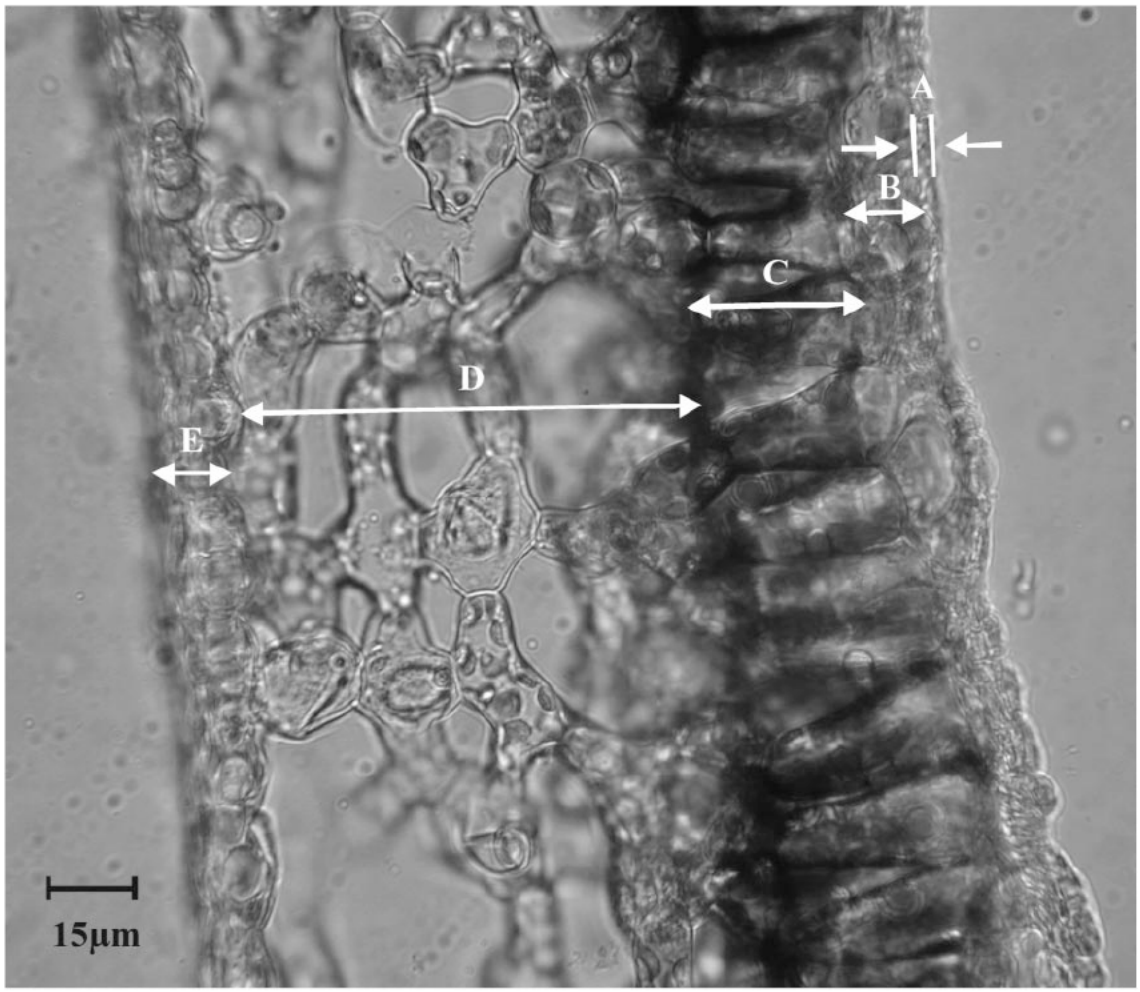

Fig. 1. Photograph (40×) of a free hand section of *Prunus serotina* (high light–low nutrient) with arrows marking measured anatomical features as follows: cuticle (A); adaxial epidermis (B); palisade mesophyll (C); spongy mesophyll (D); and abaxial epidermis (E).

tance across species, a simple Michaelis–Menten equation was fit to the data using least squares regression (PROC NLIN, SAS). Residual error from this relationship was regressed against a number of potential predictor variables including total leaf and palisade and spongy mesophyll thicknesses, LMA, tissue density, and epidermal and cuticular thickness, as well as total carotenoid concentration (PROC REG, SAS). Chlorophyll concentration was a strong predictor of both leaf reflectance and absorptance. To test for effects of species, light, and fertilization independent of changes in chlorophyll, an analysis of covariance (ANCOVA) was used where either absorptance or the reciprocal of reflectance was the continuous variable; species, fertilization, and light were the main effect terms; and the log of total chlorophyll content per area was the covariate (PROC GLM, SAS). To examine the impact of cuticular thickness on light reflected complementarily to the angle of incidence, we used the reciprocal of complementary reflectance as the dependent variable and the reciprocal of spectral reflectance and log of cuticle thickness as predictor variables in a multiple regression analysis (PROC GLM, SAS). For all analyses, the mean value for the three sampled leaves was used for each plant value resulting in three to five sample values for each species-by-treatment combination.

RESULTS

Leaf optical properties—Total leaf reflectance in the visible range differed among species with *A. rubrum* having the highest reflectance and *A. saccharum* the lowest (Table 1). Both light and soil nutrient environments influenced spectral reflectance; however, the effect of the soil nutrient environment was only marginally significant (Table 2). Total reflectance was higher for plants grown in high light and those not receiving fertilizer (Fig. 2); this response was similar across species (Table 1).

Transmittance in the visible range differed substantially across species, with *A. rubrum* leaves transmitting almost twice as much light as *Q. rubra* and *A. saccharum* (Table 1). Light treatment had no effect on transmittance; however, fertilization resulted in decreased transmittance in all species except *A. rubrum*. As a result of both high transmittance and reflectance, *A. rubrum* had the lowest spectral absorptance among species (79%), while the other four species were roughly similar (83–84%). Fertilization increased total absorptance across species by between 2–5%, whereas light treatment had no significant effect (Table 1). There was, however, a trend toward increased absorptance in the low light treatment, corresponding to decreased reflectance under low light.

Total chlorophyll concentration (Chl_{tot}) ranged from 66 µg/cm² in *A. rubrum* to 84 µg/cm² in *Q. rubra*, which closely tracks the observed species differences in absorptance (Table 1). Chl_{tot} was greater in the low light treatment for all species

TABLE 1. Leaf characteristics for high (L) and low (l) light and high (N) and low (n) nutrient treatments of five temperate deciduous tree species. Means and standard errors of untransformed data are shown (N = 3–5).

Variable	Treatment	Species				
		A. rubrum	*A. saccharum*	*F. americana*	*P. serotina*	*Q. rubra*
Reflectance (%)	Ln	9.82 ± 0.66	7.86 ± 0.74	8.92 ± 0.66	7.69 ± 0.64	9.58 ± 0.80
	LN	8.59 ± 0.85	7.27 ± 0.66	8.14 ± 0.69	7.88 ± 0.85	6.96 ± 1.04
	ln	9.31 ± 0.66	7.05 ± 0.67	8.46 ± 7.24	7.24 ± 0.89	6.74 ± 0.74
	lN	9.87 ± 0.81	6.61 ± 0.59	6.68 ± 0.66	7.07 ± 0.81	7.28 ± 0.95
Transmittance (%)	Ln	11.01 ± 1.31	7.28 ± 1.46	9.06 ± 1.31	8.98 ± 1.69	11.36 ± 1.57
	LN	9.17 ± 1.68	7.04 ± 1.37	8.07 ± 1.20	7.39 ± 1.72	4.47 ± 2.07
	ln	12.24 ± 1.21	6.84 ± 1.30	10.30 ± 1.48	9.53 ± 1.60	4.99 ± 1.45
	lN	14.28 ± 1.69	5.06 ± 1.29	6.45 ± 1.31	7.58 ± 1.69	4.04 ± 2.01
Absorptance (%)	Ln	79.17 ± 1.92	84.86 ± 2.15	82.03 ± 1.94	83.12 ± 2.48	79.06 ± 2.50
	LN	82.25 ± 2.48	85.69 ± 1.92	83.78 ± 1.97	84.73 ± 2.38	88.57 ± 3.04
	ln	78.46 ± 1.82	86.11 ± 1.90	81.24 ± 2.20	83.22 ± 2.50	88.27 ± 2.45
	lN	75.85 ± 2.49	88.33 ± 1.87	86.87 ± 1.93	85.35 ± 2.44	88.68 ± 3.10
Abs/Chl (%/mg)	Ln	15.98 ± 1.06	14.76 ± 1.22	16.88 ± 1.06	15.04 ± 1.25	18.70 ± 1.49
	LN	13.86 ± 1.22	15.95 ± 1.06	15.70 ± 1.10	15.11 ± 1.50	13.08 ± 1.45
	ln	16.52 ± 0.94	13.50 ± 0.90	16.47 ± 1.03	15.64 ± 1.22	12.93 ± 1.00
	lN	16.38 ± 1.20	12.27 ± 0.97	14.36 ± 0.89	11.76 ± 1.49	11.50 ± 1.55
Abs/Biomass (%/g)	Ln	86.66 ± 4.73	103.86 ± 4.97	124.63 ± 14.48	91.74 ± 2.11	85.29 ± 1.57
	LN	97.09 ± 6.54	105.73 ± 7.11	113.10 ± 9.94	96.84 ± 17.28	78.43 ± 1.56
	ln	127.92 ± 5.58	127.45 ± 5.57	169.28 ± 9.95	146.44 ± 10.61	105.23 ± 4.71
	lN	136.07 ± 18.36	127.82 ± 5.00	150.89 ± 6.22	138.44 ± 12.65	110.93 ± 3.47
LMA (mg/cm^2)	Ln	11.73 ± 0.37	10.78 ± 0.35	8.85 ± 0.58	11.79 ± 0.45	12.13 ± 0.46
	LN	10.92 ± 0.48	10.54 ± 0.55	9.60 ± 0.57	11.95 ± 0.87	14.37 ± 0.12
	ln	7.84 ± 0.30	8.65 ± 0.27	6.16 ± 0.19	7.31 ± 0.31	10.75 ± 0.37
	lN	7.36 ± 0.54	9.23 ± 0.37	7.38 ± 0.28	7.88 ± 0.64	10.18 ± 0.55
Tissue Density (g/cm^3)	Ln	1.24 ± 0.06	0.99 ± 0.05	1.31 ± 0.18	2.04 ± 0.08	1.18 ± 0.10
	LN	1.27 ± 0.10	0.96 ± 0.08	1.12 ± 0.07	1.86 ± 0.24	1.18 ± 0.06
	ln	0.62 ± 0.01	0.76 ± 0.05	0.67 ± 0.06	0.94 ± 0.09	1.06 ± 0.06
	lN	0.91 ± 0.13	0.78 ± 0.04	0.78 ± 0.03	1.23 ± 0.12	1.13 ± 0.01
Leaf Thickness (μm)	Ln	100.64 ± 6.83	96.07 ± 0.50	111.57 ± 9.58	170.25 ± 11.81	111.05 ± 9.12
	LN	120.47 ± 6.46	93.59 ± 6.50	116.13 ± 6.06	156.70 ± 12.26	134.72 ± 3.76
	ln	82.72 ± 1.87	81.00 ± 5.04	114.39 ± 6.28	127.31 ± 1.95	102.75 ± 8.83
	lN	103.23 ± 10.14	87.35 ± 2.65	102.50 ± 3.42	150.63 ± 18.02	93.58 ± 10.19
Cuticle Thickness (μm)	Ln	2.54 ± 0.44	2.39 ± 0.15	1.70 ± 0.23	4.36 ± 0.43	3.37 ± 0.56
	LN	2.64 ± 0.15	2.05 ± 0.12	2.54 ± 0.24	4.57 ± 0.76	4.14 ± 0.26
	ln	1.83 ± 0.05	1.88 ± 0.12	1.99 ± 0.06	3.44 ± 0.13	3.07 ± 0.35
	lN	2.50 ± 0.93	1.91 ± 0.13	2.12 ± 0.30	2.81 ± 0.01	2.82 ± 0.02
Upper Epidermis Thickness (μm)	Ln	13.68 ± 0.83	11.79 ± 0.83	11.04 ± 0.96	14.30 ± 0.91	16.59 ± 1.28
	LN	16.54 ± 1.17	10.91 ± 0.80	10.12 ± 1.02	13.52 ± 1.17	16.97 ± 1.02
	ln	12.85 ± 1.09	11.19 ± 0.87	10.54 ± 1.02	14.00 ± 1.17	16.83 ± 0.77
	lN	10.50 ± 1.44	10.66 ± 0.87	10.41 ± 0.64	11.39 ± 1.44	14.31 ± 2.03
Palisade Thickness (μm)	Ln	40.50 ± 4.23	35.98 ± 1.98	44.98 ± 5.14	51.66 ± 6.14	32.80 ± 6.16
	LN	50.49 ± 4.55	37.32 ± 4.09	38.82 ± 2.07	53.65 ± 13.63	49.53 ± 2.51
	ln	30.05 ± 3.07	30.48 ± 2.57	32.59 ± 1.82	26.28 ± 3.66	31.79 ± 2.59
	lN	31.11 ± 0.81	34.52 ± 4.83	33.19 ± 2.90	37.77 ± 6.91	34.11 ± 3.83
Spongy Mesophyll Thickness (μm)	Ln	34.99 ± 2.13	32.88 ± 3.01	49.14 ± 5.31	87.53 ± 8.32	46.00 ± 5.23
	LN	43.02 ± 5.71	33.81 ± 2.41	55.47 ± 4.73	75.87 ± 2.01	58.92 ± 3.15
	ln	26.60 ± 1.63	32.91 ± 3.93	56.75 ± 4.78	70.97 ± 0.48	41.54 ± 4.21
	lN	46.42 ± 12.01	34.25 ± 3.09	49.09 ± 3.41	76.47 ± 8.56	35.95 ± 6.06
Chl_{tot} (μg/cm^2)	Ln	63.01 ± 6.01	76.07 ± 6.94	61.78 ± 6.01	71.63 ± 6.94	59.55 ± 5.80
	LN	75.85 ± 6.94	68.71 ± 6.01	69.01 ± 6.01	70.27 ± 8.50	87.00 ± 8.53
	ln	62.09 ± 5.38	82.81 ± 5.38	63.75 ± 6.34	67.78 ± 6.94	87.72 ± 4.91
	lN	61.32 ± 6.94	93.61 ± 5.38	77.90 ± 5.38	93.20 ± 8.50	100.89 ± 12.03
Chl_a (μg/cm^2)	Ln	30.02 ± 4.66	41.44 ± 4.66	31.55 ± 4.04	40.20 ± 4.70	33.59 ± 5.71
	LN	42.28 ± 3.01	37.70 ± 4.04	36.48 ± 4.04	39.43 ± 5.70	54.78 ± 5.70
	ln	32.14 ± 4.60	46.84 ± 3.61	35.26 ± 4.24	35.63 ± 4.66	53.56 ± 3.30
	lN	32.31 ± 4.66	54.11 ± 3.61	44.46 ± 3.01	56.17 ± 5.71	59.89 ± 8.08
Chl_b (μg/cm^2)	Ln	32.99 ± 2.89	34.62 ± 3.52	30.23 ± 1.95	31.84 ± 2.63	25.96 ± 2.68
	LN	33.56 ± 1.42	31.01 ± 1.19	32.53 ± 2.21	30.84 ± 2.26	31.98 ± 0.87
	ln	29.95 ± 2.16	35.97 ± 1.81	28.49 ± 1.30	32.15 ± 2.29	34.59 ± 1.04
	lN	29.01 ± 2.23	39.50 ± 2.20	33.74 ± 1.21	37.03 ± 3.08	41.00 ± 2.83
Car_{tot} (μg/cm^2)	Ln	9.51 ± 0.41	12.42 ± 0.65	10.15 ± 0.27	11.73 ± 0.53	10.27 ± 1.26
	LN	12.04 ± 0.69	11.32 ± 0.26	11.27 ± 0.53	11.75 ± 0.36	14.50 ± 0.89
	ln	9.92 ± 0.46	13.11 ± 0.54	10.71 ± 0.39	10.79 ± 0.47	14.41 ± 0.26
	lN	9.86 ± 0.74	14.20 ± 0.55	12.44 ± 0.36	15.14 ± 1.78	15.53 ± 0.68

TABLE 2. *F* values (*P* values) for the three-way ANOVA on each leaf characteristic for five temperate deciduous tree species. Variables include percent absorbance, reflectance and transmittance (%A, %R or %T), absorption efficiency on a cholophyll (Abs/Chl) and biomass (Abs/biomass) basis, total, a and b chlorophyll (Chl_{tot}, Chl*a*, Chl*b*), carotenoids, anatomical characteristics, leaf mass per area (LMA) and tissue density.

Variable	Spp	Light	Fert	Spp × Light	Spp × Fert	Light × Fert	Spp × Light × Fert
Sqrt Arcsine %A	**6.46** (<0.0001)	0.42 (ns)	4.66 (0.0351)	1.52 (ns)	0.50 (ns)	0.57 (ns)	1.03 (ns)
Log $\%R_{total}$	**5.18** (0.0012)	**4.52** (0.0378)	**3.59** (0.0631)	0.71 (ns)	0.49 (ns)	1.72 (ns)	1.38 (ns)
Log %T	**9.51** (<0.0001)	0.46 (ns)	**9.38** (0.0034)	2.08 (0.0948)	1.10 (ns)	0.48 (ns)	1.54 (ns)
Abs/Chl	2.42 (0.0608)	**6.53** (0.0137)	**9.36** (0.0036)	**2.69** (0.0419)	1.07 (ns)	0.05 (ns)	1.63 (ns)
Abs/Biomass	**10.32** (<0.0001)	**61.74** (<0.0001)	0.16 (ns)	1.54 (ns)	1.00 (ns)	0.13 (ns)	0.16 (ns)
Chl_{tot}	**5.14** (0.0015)	**7.81** (0.0073)	**10.36** (0.0023)	2.55 (0.0503)	0.89 (ns)	0.58 (ns)	1.63 (ns)
Chl*a*	**7.31** (<0.0001)	**8.91** (0.0044)	**13.24** (0.0006)	2.04 (ns)	0.82 (ns)	0.19 (ns)	2.39 (0.0630)
Chl*b*	1.99 (ns)	**4.12** (0.0478)	3.97 (0.0519)	**2.93** (0.0299)	0.98 (ns)	1.41 (ns)	0.47 (ns)
Carotenoids	**8.70** (<0.0001)	**6.06** (0.0124)	**14.31** (0.0004)	1.93 (ns)	1.40 (ns)	0.06 (ns)	**2.82** (0.0344)
Log Leaf Thickness	**30.26** (<0.0001)	**23.24** (<0.0001)	2.69 (ns)	0.74 (ns)	1.91 (ns)	0.00 (ns)	1.88 (ns)
Log Cuticle	19.63 (<0.0001)	**11.74** (0.0013)	1.21 (ns)	0.93 (ns)	1.30 (ns)	0.83 (ns)	1.21 (ns)
Log Upper Epidermis	**15.26** (<0.0001)	0.03 (ns)	1.30 (ns)	0.87 (ns)	2.50 (ns)	0.99 (ns)	1.55 (ns)
Log Palisade Mesophyll	0.95 (ns)	**29.02** (<0.0001)	**4.42** (<0.0410)	1.49 (ns)	1.09 (ns)	0.01 (ns)	1.19 (ns)
Log Spongy Mesophyll	**36.69** (<0.0001)	3.97 (0.0523)	2.68 (ns)	1.30 (ns)	1.89 (ns)	0.04 (ns)	1.85 (ns)
Log Lower Epidermis	**11.85** (<0.0001)	1.14 (ns)	0.00 (ns)	1.97 (ns)	0.75 (ns)	1.44 (ns)	1.91 (ns)
LMA	**23.44** (<0.0001)	**163.8** (<0.0001)	2.16 (ns)	**4.74** (0.0011)	1.85 (ns)	0.11 (ns)	1.30 (ns)
Tissue Density	**9.98** (<0.0001)	**36.68** (<0.0001)	2.06 (ns)	1.64 (ns)	0.49 (ns)	0.28 (ns)	0.67 (ns)

Note Spp, species; fert, fertilizer; ns, not significant.

except *A. rubrum* for which it decreased. Fertilization generally increased Chl_{tot} across species and light treatments. There were also large interspecific differences in chlorophyll *a* and total carotenoid concentrations but no significant differences in chlorophyll *b* (Table 2). Chlorophyll *a* (Chl *a*) concentration increased under low light across species with the exception of *A. rubrum* for which Chl *a* decreased slightly. Fertilization also resulted in increased Chl *a* concentration across species. The three-way interaction term was marginally significant. Fertilization did not affect *A. rubrum* under low light, whereas *P. serotina* failed to respond to fertilization under high light. Chl *a* increased in the other three species with fertilization, regardless of light environment. Chlorophyll *b* (Chl *b*) concentrations also increased under both the low light and high nutrient treatments (Table 1). Species differed in their response to light; both *A. rubrum* and *F. americana* had slight decreases in Chl *b*, while the other three species had higher Chl *b* under low light. Total carotenoid (Car_{tot}) concentrations were greater for saplings in low light (Table 2) and with fertilization. Species response to fertilization differed substantially across light treatments ($P = 0.0344$, Table 2).

Chlorophyll concentration and spectral absorptance had a strong asymptotic relationship ($P < 0.0001$, Fig. 3A). At low Chl_{tot}, absorptance increased fairly rapidly, then saturated at greater concentrations. Species positions were distinct along the curve with *A. rubrum* and *P. serotina* at the lower end, *F. americana* in the middle and *A. saccharum* and *Q. rubra* at the saturating portion of the relationship (Fig. 3A). The reverse pattern held for reflectance ($P < 0.0001$, Fig. 3B). In an analysis of covariance on data that had been transformed to linearize relationships, species differed significantly in their relationships between both absorptance ($P < 0.0001$) and reflectance ($P < 0.0001$) as a function of chlorophyll concentration. The light treatment still strongly affected leaf reflectance when the influence of chlorophyll was removed ($P < 0.0001$), as did fertilization but to a lesser extent ($P = 0.0016$). Independent of chlorophyll, light was still nonsignificant ($P = 0.1738$), whereas fertilization substantially influenced spectral absorptance ($P < 0.0001$). The interaction term between chlorophyll and light treatment was significant ($P = 0.0005$), which could explain the nonsignificant trend toward increased absorptance in the low light treatment (Table 1).

Saplings in the high light treatment generally had greater absorption efficiency on a chorophyll basis (Abs/chl), but this differed across species (Fig. 4A, Table 2). For example, *A. rubrum* had greater Abs/Chl in low light, while *F. americana* shifted its Abs/Chl very little with light environment (Fig. 4A). Fertilization generally decreased Abs/Chl across species and treatments although this decrease was fairly small in some cases (Table 1). On a biomass basis, however, absorption efficiency increased by approximately 40% in the low light treatment, while fertilization had no effect on Abs/Biomass (Fig. 4B, Table 1). *Acer saccharum* had the least plasticity in its Abs/Biomass, similar to many of the other optical and anatomical traits measured for this species (Table 1). Abs/Biomass increased the most in *P. serotina* and *A. rubrum* with increased light availability (Fig. 4B, Table 1).

Spectral reflectance patterns—In all species except for *Q. rubra*, light and fertilization resulted in an additive optical response. For *P. serotina*, *A. saccharum* and *F. americana*, plants grown in the high light–low nutrient combination had the greatest leaf reflectance and those in the low light–high nutrient combination the least. Plants grown in high light–high nutrient and low light–low nutrient environments were most similar in their reflectance response across wavelengths (Fig. 2). *Acer rubrum* showed the reverse pattern. Across species, reflectance differences were most obvious in the green spectrum near 550 nm and the red spectrum near 700 nm and were fairly small between 400–450 nm and near the 680 nm chlorophyll peak (Fig. 2).

Leaf anatomy—With the exception of the palisade mesophyll, all leaf anatomical characteristics varied substantially across species (Fig. 5, Tables 1 and 2). Treatment effects were variable with light availability affecting several anatomical characteristics, whereas fertilization only significantly affected palisade mesophyll thickness. Total leaf thickness varied substantially from 90–140 μm across species, with *P. serotina* having the thickest leaves and *A. saccharum* the thinnest (Fig.

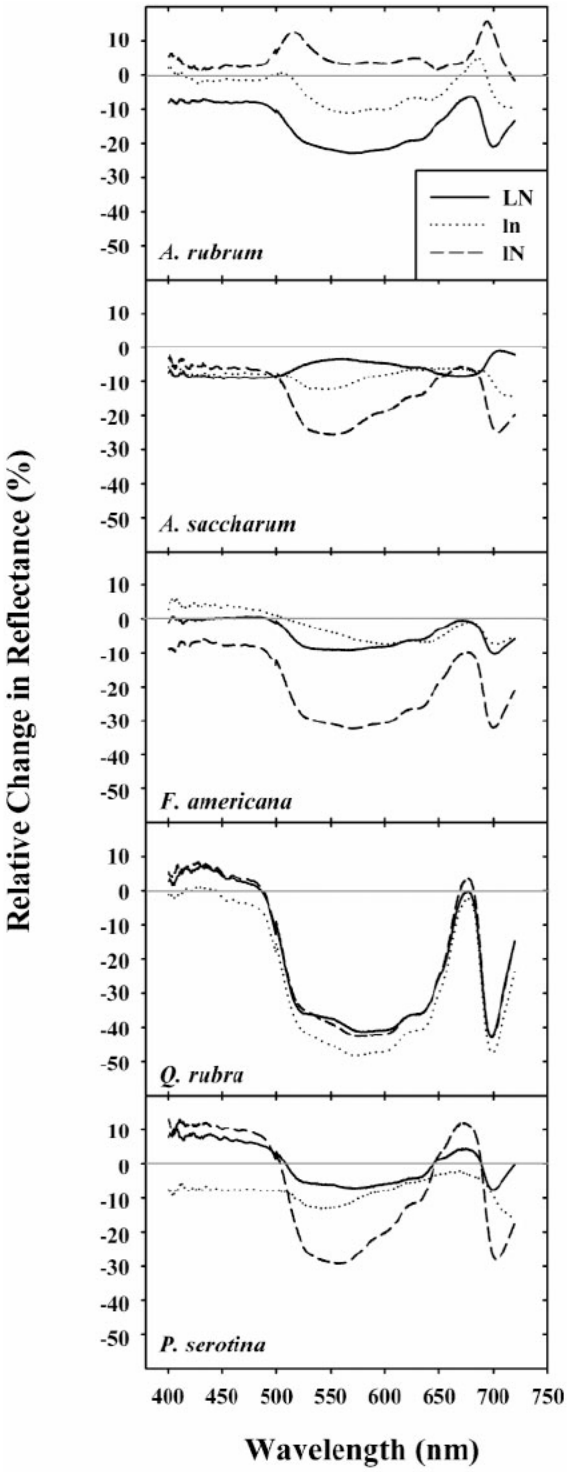

Fig. 2. Sensitivity of leaf reflectance to changes in light and nutrient availability calculated as $(R_{trt} - R_{control})/R_{control}$. Control treatment was Ln (high light, low nutrients). Treatment abbreviations are as follows: (L = high light; l = low light; N = high nutrients; n = low nutrients). As instrument noise was high in the wavelengths below 500 nm, the data present a moving average in this region.

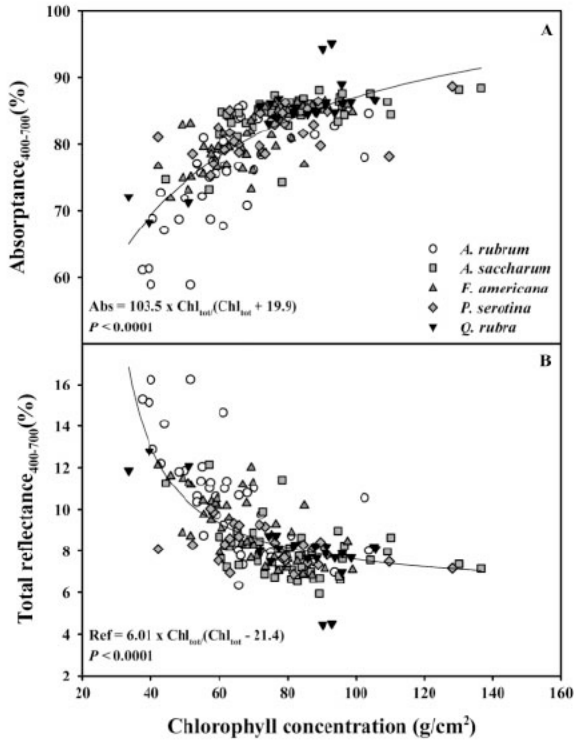

Fig. 3. Relationship of leaf spectral absorptance (A) and reflectance (B) to total chlorophyll concentration across species and treatments.

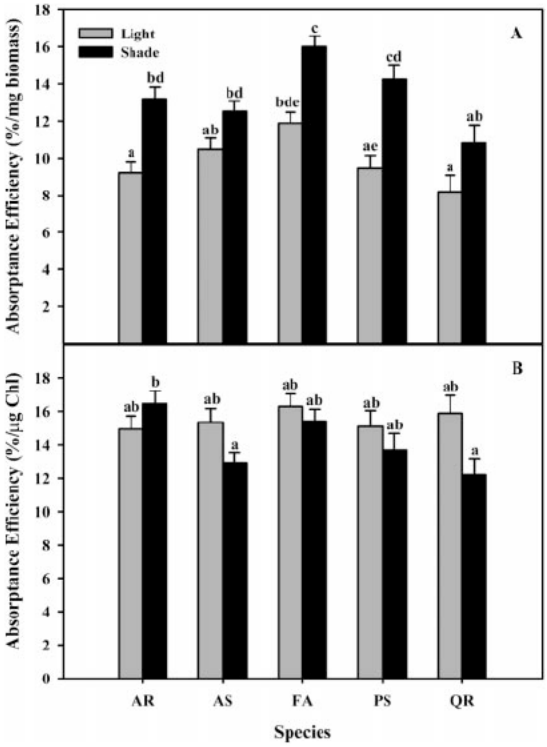

Fig. 4. Mean (±SE) absorption efficiency on biomass (A) and chlorophyll (B) basis for five temperate deciduous tree species grown in high and low light. AS, *Acer saccharum*; AR, *A. rubrum*; FA, *Fraxinus americana*; PS, *Prunus serotina*; and QR, *Quercus rubra*. Pairwise comparisons are across species and treatment.

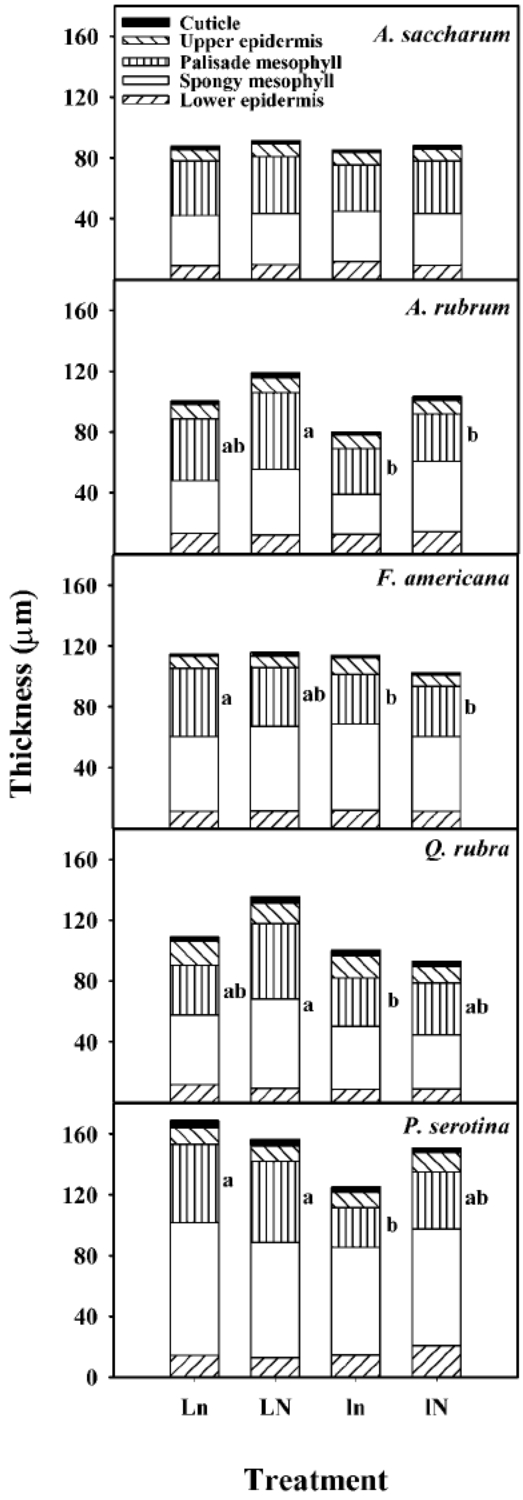

Fig. 5. Mean leaf anatomical characteristics for five temperate tree species exposed to different light and nutrient availabilities (L = high light; l = low light; N = high nutrients; n = low nutrients). Average standard errors in μm for different tissue layers are as follows: 0.27 (cuticle), 0.83 (upper epidermis), 4.17 (palisade mesophyll), 4.51 (spongy mesophyll) and 0.97 (lower epidermis).

5, Table 2). Leaves in the high light treatment were consistently thicker than those in the low light treatment, whereas fertilization had no significant effect on total leaf thickness. There was an overall trend across species of increased cuticle thickness under high light ($P = 0.0013$) with *Q. rubra* and *P. serotina* having the greatest increases with increased light (Fig. 5, Table 2). While fertilization did not significantly affect cuticle thickness, four of five species had the highest cuticular thickness in the high light–high nutrient treatment (Table 1). Adaxial and abaxial epidermal thicknesses were consistent across treatments. Palisade mesophyll thickness varied with both light and nutrient availability. Overall, palisade layer thickness was greater in the high light and high nutrient treatments (Fig. 5). In contrast to the palisade mesophyll, spongy mesophyll thickness varied substantially across species but showed very little response to either light or fertilization (Fig. 5).

Leaf mass per area and tissue density—Leaf mass per area (LMA) ranged from 6.2 mg/cm^2 in *F. americana* to 12.1 mg/cm^2 in *Q. rubra* and differed significantly among species (Tables 1 and 2). Plants grown in high light had greater LMA than those grown in low light but the magnitude of the response differed among species. *Acer saccharum* increased its LMA by only 19% in the high light treatment, whereas *P. serotina* increased its LMA by 57% under a high light regime. Fertilization had no effect on LMA. Leaf tissue density responded similarly to LMA with increases up to 75% in the high light treatment (Table 1), a response that was consistent across species (Table 2). Fertilization also had no effect on tissue density (Table 2).

Leaf optical parameters as a function of other leaf traits—When the residuals of the absorptance or reflectance and chlorophyll relationship were regressed against other leaf characteristics, none of the anatomical features, LMA, or tissue density predicted residual variation in either leaf absorptance or reflectance. However, regression analyses indicated that total carotenoid concentration contributed significantly to the residual variation in both leaf absorptance ($P = 0.0418$) and reflectance ($P = 0.0011$). Total carotenoid concentration explained an additional 2.3% of the variation in absorptance and 5.7% of reflectance. Multiple regression analyses of the transformed reflectance data indicated that cuticular thickness contributed significantly to the variation in light reflected at an angle complementary to the angle of incidence ($P = 0.0323$).

DISCUSSION

Leaf optical parameters showed large, yet species-specific responses to light and nutrient availability. Pigment concentrations, primarily total chlorophyll but also carotenoids, were good predictors of patterns of both spectral reflectance and absorptance. Light was the more influential resource affecting spectral reflectance, and its effects were primarily a function of changes in chlorophyll concentration, although increased cuticular thickness did increase light reflected at a complementary angle to the angle of incidence. Fertilization had larger effects on spectral absorptance than did light. However, light availability strongly influenced absorption efficiency on a biomass basis with 40% greater efficiency in the low light treatment, due to increased LMA. Absorptance increased by up to 7% in the low light treatment with fertilization, suggesting a

potentially important role of nutrient availability to low light carbon balance.

Stress vs. resource availability effects on leaf optics—Several studies have provided strong evidence that plants respond to acute stress with increased spectral reflectance (Bornman and Vogelmann, 1991; Carter, 1993; Carter et al., 1995, 2000), with the most predictable increases in the green (~550 nm) and the red (~700 nm) wavelengths. Carter (1993) determined that increased reflectance in these ranges was consistent across a number of biotic and abiotic stress agents and species and suggested that remote sensing within these spectrally narrow ranges may allow detection of plant stress in densely vegetated landscapes. Our data show, however, that unstressed plants exposed to moderate changes in resource availability had large optical responses, similar in magnitude and spectral range to their responses to acute stress. The difference maxima in our data were centered on 550 and 700 nm and the minima in the violet region (400–450 nm) and around the chlorophyll peaks (~670 nm), nearly identical responses to acute stress. In the present study, reflectance differed by between 5% and 50% across light and nutrient treatments at the sensitivity maxima. Carter (1993) found differences ranging from 20% to 160%; however, responses to five out of eight stress agents fell within the range of changes reported in the present study. Only leaf senescence, pathogen infection and inadequate mycorrhizal inoculation resulted in proportional reflectance changes greater than 50%. Plants are likely to experience temporal and spatial variation in light and nutrient availability similar in magnitude to those used in the present study, which could result in substantial differences in spectral reflectance patterns between vegetated areas similar to those produced by acute plant stress. Our findings thus put into question the use of reflectance changes in the visible spectrum as adequate indicators of abiotic stress factors in the absence of extrinsic information on the stresses themselves.

Leaf pigments as predictors of leaf optical properties—Patterns of both spectral reflectance and absorptance were primarily driven by chlorophyll concentration, a pattern similar to findings of several other studies (Thomas and Gausman, 1977; Agustı́et al., 1994; Gitelson and Merzlyak, 1994). As chlorophyll density increases, the efficiency of light capture by any given chlorophyll molecule decreases. Agustı́et al. (1994) found this relationship held across a range of photosynthetic organisms from single-celled cyanobacteria to trees and is due to effects of internal shading when chlorophyll concentrations are high within the leaf. In the present study, changes in total reflectance with both increased light and nutrient availability were primarily a result of altered chlorophyll concentration. However, when the effect of chlorophyll was removed, both light and fertilization still predicted patterns of spectral reflectance, and fertilization still explained variation in the absorptance data, indicating that factors other than chlorophyll were contributing to observed changes in leaf optical properties.

Total carotenoid concentration also contributed significantly to observed variation in both spectral absorptance and reflectance, explaining 2.3% and 5.7% of the variation respectively. Carotenoids strongly absorb light in the blue region of the spectrum (Palett and Young, 1993), particularly in the 500–520 nm region (Gitelson et al., 1966; Zur et al., 2000), and their concentrations are generally second only to the cholorophylls. Therefore once the influence of chlorophyll is removed, the impact of carotenoid concentration on leaf optical properties should be detectable, as was evident in the present study.

While chlorophyll concentration was the main determinant of both spectral reflectance and absorptance, species differed substantially in their relationships of either reflectance or absorptance as a function of chlorophyll concentration. Leaf anatomy is expected to contribute to variation in tissue optical properties. We were not, however, able to detect a direct influence of the anatomical traits measured, despite large differences among treatments and species. It may be that we were not measuring the right traits, either optical or anatomical, to detect this relationship. Leaf optical properties may be indirectly affected by changes in leaf anatomy through their important role in the determination of light distribution within the leaf (Vogelmann et al., 1996). For example, convex epidermal cells can act to collect and focus light, which may increase the probability of interception of photons by chloroplasts (Bone et al., 1985; Myers et al., 1994); the convexity of epidermal cells varied among species in the present study (personal observation), which could alter the focusing properties without necessarily changing epidermal dimensions. Additionally, palisade mesophyll cell shape may alter light penetration within the leaf, changes in spongy mesophyll cell shape can affect optical path length, and leaf anatomy influences chloroplast distribution; all of which could affect leaf optical properties (Terashima and Saeki, 1983; Vogelmann and Martin, 1993; DeLucia et al., 1996). Additionally, internal cellular structure, specifically air-cell interfaces, are thought to be particularly important in determining reflectance in the NIR region (700–1300 nm) due to refractive differences between hydrated cells and intercellular air spaces, which cause backscattering of light, in addition to the weak absorptance of NIR by leaves (Knipling, 1970; Slaton et al., 2001). The large anatomical changes observed may therefore have a stronger influence on bulk leaf optical properties outside of the visible range.

We did find that cuticle thickness significantly influenced the amount of light reflected at an angle complementary to the angle of incidence. It has been previously suggested that increased cuticle thickness, under high light conditions, may be influential in the reception and redistribution of radiant energy through reflection away from plant tissue at the air-cuticle interface (Cameron, 1970; Baker, 1982; Grant, 1987; Grant et al., 1993). Our multiple regression analysis showed that leaf cuticle thickness was able to predict a significant amount of variation in the complementarily reflected light across species and treatments, providing further evidence for the importance of the leaf cuticle in determining leaf reflectance patterns. Cuticle thickness was thus the only anatomical characteristic measured that significantly correlated with leaf optical parameters in the visible range.

Efficiency of light capture—Absorptance was not strongly affected by light availability but increased greatly in response to fertilization. This contradicts the hypothesis that shade leaves should be capable of absorbing a higher proportion of incident radiation as a response to light limitation (Björkman, 1981; Givnish, 1984). Several other studies have shown similar patterns: Poorter et al. (2000) found slightly higher spectral absorptance in sun than shade leaves of five tropical species. Survey studies of both tropical (Lee and Graham, 1986;

Cao, 2000) and temperate (Knapp and Carter, 1998) species found no relationship between light environment and leaf absorptance. In the present study, although there was a nonsignificant trend toward increased absorptance in the low light treatment, differences between sun and shade plants only became apparent when absorptance was expressed on a biomass basis, which reflects the cost-efficiency of the investment in photosynthetic tissue (Agustı et al., 1994). Per unit biomass investment, plants in the low light environment absorbed 20–50% more incident radiation than did high light grown plants. Efficient light capture per unit biomass should contribute to positive carbon balances at lower irradiances given the reduction in metabolic costs compared to thicker tissues as well as the reduced investment in photosynthetic tissue construction. Plants in the high light treatment generally had thicker leaves and greater LMA, a pattern that is well documented in the literature (Boardman, 1977; Björkman, 1981) in addition to greater tissue density. Shifts in absorption efficiency were primarily being driven by changes in LMA, providing additional evidence for the importance of LMA in plant responses to the light environment.

The observed response of leaf absorptance to fertilization, which was particularly evident under low light conditions, may be of consequence to whole-plant carbon gain. Fertilization increased leaf spectral absorptance by as much as 7% in the low light treatment, a substantial contribution to light capture. This was achieved primarily through additional allocation of resources to chlorophyll when nutrients were more readily available. In low light environments, it is generally assumed that plants are most strongly limited by and responsive to light availability and that they will respond strongly to belowground resources only once light limitation is removed. However, various measures of the efficiency of light use, such as quantum yield and spectral absorptance, have both been shown to be fairly consistent between high and low light environments (Björkman, 1981; Lee et al., 1990; Knapp and Carter, 1998; Poorter et al., 2000); our study similarly found no absorptance response to light availability. The present findings suggest, however, that plant responses to nutrient availability may play a greater role in light limited environments than previously acknowledged through their contribution to light harvesting capabilities. Temporal and spatial heterogeneity in nutrient availability in light limited environments, such as the forest understory, may thus contribute to sapling survival because an increase in absorptance capacity could make the difference between a positive or negative carbon balance for a plant occurring in a light environment near its whole plant compensation point. Differential species responses to fertilization suggest that certain species may be better able to take advantage of changes in nutrient availability, which could also play an important role in regeneration dynamics in the forest understory as species better able to utilize moderate increases in nutrient availability to enhance spectral absorptance may more frequently achieve a positive carbon balance in light limited environments.

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